

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims

All pending claims are cancelled.

Please add new claims 41-89 as follows:

41. (New) A method for the transformation of plastid genomes of a plant species, comprising the steps of:

- a) providing a transformation vector carrying a DNA sequence of interest;
- b) subjecting a plant material, which comprises plastids, to a transformation treatment in order to allow the plastids to receive the transformation vector;
- c) placing the thus treated plant material for a period of time into contact with a culture medium without a selection agent;
- d) subsequently placing the plant material into contact with a culture medium comprising a selection agent; and
- e) refreshing the culture medium comprising a selection agent to allow plant material comprising plastids that have acquired the DNA of interest to grow into transformants.

42. (New) The method as claimed in claim 41, wherein the plant species is *Asteraceae*.

43. (New) The method as claimed in claim 41, wherein the expression vector comprises:

- an expression cassette which comprises optionally a promoter active in the plant species to be transformed, a DNA insertion site for receiving the transforming DNA of interest, optionally one or more selection markers conferring a selectable phenotype on cells having plastids that are transformed with the expression cassette, and optionally a DNA

sequence encoding a transcription termination region active in the plant species to be transformed,

- optionally a set of DNA targeting segments located on either side of the expression cassette that allow double homologous recombination of the expression cassette with the plastic genome of interest, and

- optionally a DNA sequence encoding a gene of interest inserted into the insertion site of the expression cassette.

44. (New) The method as claimed in claim 43, wherein the vector comprises the promoter, the DNA sequence encoding the gene of interest, the one or more selection markers and the set of DNA targeting segments.

45. (New) The method as claimed in claim 41, wherein the transformants carry the DNA of interest in their genome.

46. (New) The method as claimed in claim 41, wherein the plastids to be transformed are selected from the group consisting of chloroplasts, amyloplasts, elaioplasts, etioplasts, chromoplasts, leucoplasts and proplasts.

47. (New) The method as claimed in claim 42, wherein the promoter is selected from the group consisting of the chloroplast specific ribosomal RNA operon promoter *rrn*(16S rRNA), *psbA*, *rbcL*, *trnV* and *rps16*.

48. (New) The method as claimed in claim 41, wherein the DNA of interest is a gene encoding a therapeutic or prophylactic (bio)pharmaceutical (poly)peptide, such as an edible vaccine.

49. (New) The method as claimed in claim 41, wherein the DNA of interest is selected from the group consisting of genes encoding herbicide resistance, insect resistance, fungal resistance, bacterial resistance; genes that lead to stress tolerance, for

instance to cold, high salt or minerals; and genes that improve yield, starch accumulation, fatty acid accumulation or photosynthesis.

50. (New) The method as claimed in claim 42, wherein the terminator is selected from the group consisting of the *psb A* termination sequence, *rrn*, *rbcL*, *trnV* and *rps16*.

51. (New) The method as claimed in claim 42, wherein the selection marker is a gene conferring resistance against agents selected from the group consisting of spectinomycin, streptomycin, kanamycin, hygromycin and chloramphenicol, glyphosate and bialaphose.

52. (New) The method as claimed in claim 42, wherein the selection marker is a visual marker, such as a fluorescent marker like *gfp* (green fluorescence protein).

53. (New) The method as claimed in claim 52, wherein steps d) and e) of the transformation method are omitted and the transformants are selected by illuminating the putative transformants with an appropriate light source corresponding to the visual marker and selecting the plant material that shows fluorescence.

54. (New) The method as claimed in claim 42, wherein the DNA segments that allow double homologous recombination of the DNA of interest with the plastid genome of interest have a DNA sequence that is homologous to a part of the plastid genome.

55. (New) The method as claimed in claim 54, wherein the set of DNA segment is selected from the group consisting of the *trnI (oriA)/trnA* region and the 16S/*trnV*/ORF70B region of a lettuce chloroplast genome.

56. (New) The method as claimed in claim 54, wherein the set of DNA segments is selected from LCV1 A-B and LVC1 C-D, and LCV2 A-B and LCV2 C-D.

57. (New) The method as claimed in claim 41, wherein the transformation treatment is selected from the group consisting of electroporation, particle gun transformation, polyethylene glycol transformation and whiskers technology.

58. (New) The method as claimed in claim 41, wherein the transformation treatment is polyethylene glycol transformation and the period of time during which the treated plant material is placed into contact with a culture medium without selection agent is 1 to 14 days.

59. (New) The method as claimed in claim 41, wherein the transformation treatment is polyethylene glycol transformation and the period of time during which the treated plant material is placed into contact with a culture medium without selection agent is 3 to 7 days.

60. (New) The method as claimed in claim 41, wherein the transformation treatment is polyethylene glycol transformation and the period of time during which the treated plant material is placed into contact with a culture medium without selection agent is about 6 days.

61. (New) The method as claimed in claim 41, wherein the transformation treatment is particle gun transformation and the period of time during which the treated plant material is placed into contact with a culture medium without selection agent is 1 to 14 days.

62. (New) The method as claimed in claim 41, wherein the transformation treatment is particle gun transformation and the period of time during which the treated plant material is placed into contact with a culture medium without selection agent is 1 to 5 days.

63. (New) The method as claimed in claim 41, wherein the transformation treatment is particle gun transformation and the period of time during which the treated plant material is placed into contact with a culture medium without selection agent is about 2 days.

64. (New) The method as claimed in claim 41, wherein the plant material to be treated is selected from the group consisting of plant tissue, separate cells, protoplasts and separate plastids.

65. (New) The method as claimed in claim 41, wherein the culture medium comprising the selection agent is a liquid medium.

66. (New) The method as claimed in claim 41, wherein step c) is performed in the dark.

67. (New) A vector for the transformation of plastid genomes of a plant species, said vector comprising:

- an expression cassette which comprises optionally a promoter active in the plastids of the plant species to be transformed, a DNA insertion site for receiving the transforming DNA of interest, optionally one or more selection markers conferring a selectable phenotype on cells having plastids that are transformed with the expression cassette, and optionally a DNA sequence encoding a transcription termination region active in the plastids of the plant species to be transformed, and

- optionally a set of DNA targeting segments located on either side of the expression cassette that allow double homologous recombination of the expression cassette with the plastid genome of interest.

68. (New) The vector as claimed in claim 67, wherein the plant species is *asteraceae*.

69. (New) The vector as claimed in claim 67, wherein the vector comprises the promoter, the one or more selection markers and the set of DNA targeting segments.

70. (New) The vector as claimed in claim 67, wherein said vector comprises:

- an expression cassette which comprises a promoter active in the plant species to be transformed, a DNA insertion site for receiving the transforming DNA of interest, one or more selection markers conferring a selectable phenotype on cells having plastids that are transformed with the expression cassette, and optionally a terminator active in the plant species to be transformed, and

- a set of DNA targeting segments located on either side of the expression cassette that allow double homologous recombination of the expression cassette with the plastid genome of interest.

71. (New) The vector as claimed in claim 67, further comprising a DNA sequence of interest inserted into the insertion site of the expression cassette.

72. (New) The vector as claimed in claim 67, for use in the method as claimed in claim 41.

73. (New) The vector as claimed in claim 67, wherein the promoter is selected from the group consisting of the chloroplast specific ribosomal RNA operon promoter *rrn* (16S rRNA), *psbA*, *rbcL*, *trnV* and *rps 16*.

74. (New) The vector as claimed in 67, wherein the DNA of interest is a gene encoding a therapeutic or prophylactic (bio)pharmaceutical (poly)peptide, such as an edible vaccine.

75. (New) The vector as claimed in claim 67, wherein the DNA of interest is selected from the group consisting of genes encoding herbicide resistance, insect resistance, fungal resistance, bacterial resistance; genes that lead to stress tolerance, for instance to cold, high salt or minerals; and genes that improve yield, starch accumulation, fatty acid accumulation or photosynthesis.

76. (New) The vector as claimed in claim 67, wherein the terminator is selected from the group consisting of the *psb* A termination sequence, *rrn*, *rbcL*, *trnV* and *rps16*.

77. (New) The vector as claimed in claim 67, wherein the selection marker is a gene conferring resistance against agents selected from the group consisting of spectinomycin, streptomycin, kanamycin, hygromycin and chloramphenicol, glyphosate and bialaphos.

78. (New) The vector as claimed in claim 67, wherein the selection marker is a visual marker, such as fluorescent markers like *gfp* (green fluorescence protein).

79. (New) The vector as claimed in claim 67, wherein the DNA segments that allow double homologous recombination of the DNA of interest with the plastid genome of interest have a DNA sequence that is homologous to a part of the plastid genome.

80. (New) The vector as claimed in claim 79, wherein the set of DNA segments is selected from the group consisting of the *trnI* (*oriA*)/*trnA* region and the 16S/*trnV*/ORF70B region of a lettuce chloroplast genome.

81. (New) The vector as claimed in claim 79, wherein the set of DNA segments is selected from LCV1 A-B and LCV1 C-D, and LCV2 A-B and LCV2 C-D.

82. (New) A transplastomic plant or plant part obtainable by the method as claimed in claim 41.

83. (New) A transplastomic plant or plant part transformed by the vector of claim 79, and obtainable by the method as claimed in claim 41.

84. (New) The transplastomic plant or plant part as claimed in claim 82, wherein the plant is a lettuce plant.

85. (New) The vector as claimed in claim 83, wherein the set of DNA segments is selected from the group consisting of the *trnI* (*oriA/trnA* region and the 16S/*trnV*/ORF70B region of a lettuce chloroplast genome.

86. (New) A progeny of a plant or plant part as claimed in claim 82, carrying plastids at least part of which have the gene of interest in their genome.

87. (New) A progeny of a plant or plant part as claimed in claim 83, carrying plastids at least part of which have the gene of interest in their genome.

88. (New) Plant parts as claimed in claim 82, which plant parts are selected from the group consisting of tissues, cells, meristems, calli, protoplasts, plastids, proplastids and plastid DNA.

89. (New) Plant parts as claimed in claim 83, which plant parts are selected from the group consisting of tissues, cells, meristems, calli, protoplasts, plastids, proplastids and plastid DNA.